Spectroscopy Tools to Measure Protein Folding: Correlating Structure and Function

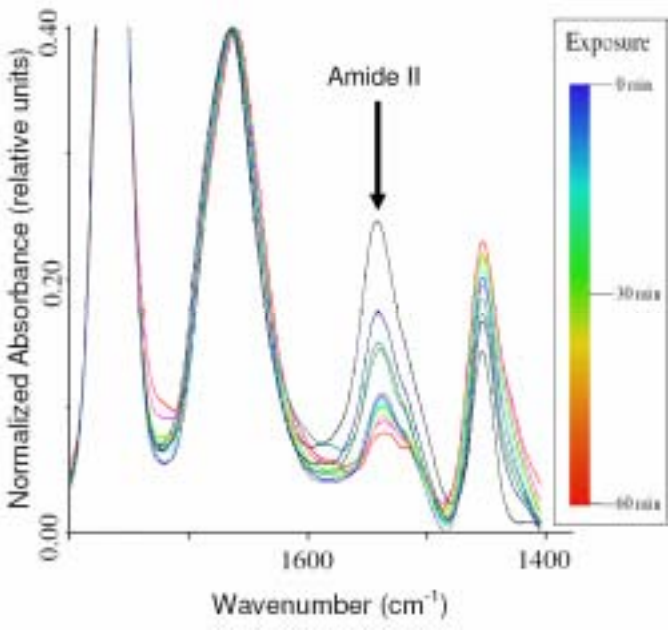
A new method is being developed to measure how individual sections of proteins (called "domains") are spatially oriented within these macromolecules. Proteins are synthesized in the cell as linear chains of amino acids and modified into distinct functional domains. The ability to measure domain spatial orientation will enable the biomanufacturing of more consistent protein-based drugs, diagnostic reagents and standards.

C. W. Meuse (Div. 831), J. B. Hubbard (MSEL)

Biopharmaceuticals, protein arrays, proteins for research purposes, and protein standards must have well characterized molecular compositions and conformations to allow comparisons of treatment protocols, disease diagnosis, etc. In collaboration with the FDA and the New York Academy of Sciences, NIST hosted a meeting, in December of 2005, entitled ***"Follow-on Biologicals: Scientific Issues in Assessing the Similarity of Follow-on Protein Products"*** to discuss the measurements that can be used to characterize proteins. A particular theme of this meeting was to identify techniques that can distinguish between measuring molecular averages and the distribution of properties such as glycoform and conformations.

NIST is responding to a need for better techniques to measure the 3-dimensional structure of proteins in their native state, to support innovation of bio-generic drugs

NIST is developing a method to obtain more information about molecular conformational distributions using spectroscopy. This will allow us to distinguish between an ensemble of molecules with only a limited number of molecular conformations and an ensemble of molecules with an extended distribution of conformations that happen to have the same average values. We form a P2 orientation order parameter that depends not only on an average orientation angle, θ, but also on the deviation from that average, i.e., the width of the orientation distribution, δθ. Comparisons of δθ, obtained from the analysis of internal reflection optical measurements on self-assembled alkanethiol monolayers tethered to gold surfaces with different amounts of order and with parameters derived from a molecular dynamics simulation of the same systems show good agreement. In FY2006, we studied the hydrogen deuterium exchange properties of several protein monolayer-on surface-systems to allow comparisons of our parameter between protein monolayer samples with different degrees of order. The results of our hydrogen deuterium exchange experiments further suggest that our, δθ, parameter is measuring conformational heterogeneity. This seems reasonable based on the idea that there can be no single molecular orientation when dealing with nonspecifically adsorbed flexible protein molecules, i.e., each protein has its own orientation.



NIST researchers have made measurements of deuterium exchange for hydrogen several anisotropic films of proteins. Our results indicate that exchange measurements can be generalized to describe protein conformational changes in protein monolayers.

The spectral changes in the infrared region of the amide group in a monolayer of bovine serum albumin (BSA) adsorbed to the surface of poly(d,l-lactic acid) and subsequently exposed to deuterated 0.01 M sodium phosphate buffer over the course of 1 h. The spectra are color-coded by exposure time according to the key (inset). The dark blue spectrum corresponds to the non-deuterated case, while the red spectrum corresponds to 1 h exposure. The thickness of the adsorbed BSA layer the films that produced these spectra, as measured via ellipsometry, ranged from (3.0 to 6.9) nm. Spectra are the averaged results of 3 experimental trials.

Future Plans

In FY07, we will extend our studies of the quantitation of protein structural heterogeneity to include different proteins and protein systems such as fibronectin on polymer surfaces, crystallizing RNase and bacteriorhodopsin undergoing its proton pumping photocycle.

Publications:

Dennis P. McDaniel, Gordon A. Shaw, John T. Elliott, Kiran Bhadriraju, Curtis W. Meuse, Koo-Hyun Chung, and Anne L. Plant **"The stiffness of collagen fibrils influences vascular smooth muscle cell phenotype"** Accepted by *Biophysical Journal*.

Jack R. Smith, Marcus T. Cicerone, Curtis W. Meuse **"Measuring Hydrogen Deuterium Exchange in Protein Monolayers"** Submitted to *Applied Spectroscopy*.

Jack R. Smith, Marcus T. Cicerone, Curtis W. Meuse **"Tertiary Structure Changes in Albumin Upon Surface Adsorption Observed Via Fourier Transform Infrared Spectroscopy"** Submitted to *Biomaterials*.